Phytochemical screening, DPPH free radical scavenging activity and FT-IR spectral analysis of *glycosmis cochinchinensis* an Important Medicinal Plant

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Abstract

Glycosmis cochinchinensis has long been used as a traditional medicine and leaf samples were randomly collected and extracted with various solvents. The extracts were chemically analyzed by fourier transform infrared spectroscopy (FT-IR) spectrometry and free radical scavenging activities were assessed by the 2,2-diphenyl-1- picrylhydrazyl (DPPH) assay. Phytochemical screening of the plants showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars. The *glycosmis cochinchinensis* scavenging activities of leaves were similar and comparable to that of BHT, the standard antioxidant used. This study, has to some extent, validated the medicinal potential of the leaves *glycosmis cochinchinensis.*

Keywords: Antioxidant, FT-IR, DPPH assay, phytochemical analysis, *Glycosmis ochinchinensis*

1. Inroduction

Plants contain a wide variety of chemical compounds broadly classified as primary metabolites, and secondary metabolites. Phytochemical characterization of plant material is important and it relates to the therapeutic actions. Natural products are believed to be an important source of new chemical substances with potential therapeutic applicability. Therefore phytochemical evaluation of plant is essential to find out the relationship between the biological activity and the chemical structure of the biologically active phytochemicals. The medicinal importance of plants play role in a some chemical substances that create a specific physiologic

action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. It is normally established that in a condition of oxidative stress, Reactive Oxygen Species (ROS) such as superoxide (O2), hydroxyl (OH-) and peroxyl radicals are generated. The ROS play a main role in the pathogenesis of different serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation.[1-3]. Antioxidant compounds in food play a main role as a health caring factor. Scientific support suggests that antioxidants decrease the danger for chronic diseases including cancer and heart diseases[4-6]. Therefore presently, the research importance is focused on the potential role of antioxidant in the action and prevention of above diseases. The most commonly used antioxidants at present are Butylated hydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT), Propyl Gallate (PG) and Tert-Butyl Hydro Quinone (TBHQ), Ascorbic acid (vitamin-c). However, they are suspected of being dependable for liver damage and carcinogenesis in laboratory animals[7]. Therefore, the growth and use of more helpful antioxidants of natural origin are most wanted. Flavonoids are effective watersoluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protects in opposition to all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart diseases[8].

Figure 1 : Glycosmis cochinchinensis plant

Glycosmis cochinchinensis (Lour) Pierre, belonging to Rutaceae family, is distributed throughout the Indian region. The plants in this genus generally produce a variety of alkaloids including acridones, quinolones, quinazolines, furoquinolines, carbazoles, indoles and amide derivatives[9-11] . Many of these exhibited interesting pharmacological properties such as antioxidant, anti-HIV, antifungal, antitumor promoter and cytotoxicities . In this paper, we present the results of the antioxidant activities of methanol, ethanol and water extracts of the leaves and stems of *Glycosmis cochinchinensis*. The findings from this work may add to the overall value of the medicinal potential of the herb.

2. Materials and Methods

Fresh leaves of *glycosmis cochinchinensis* were collected from the annamalai university Campus, chidambaram, Tamil Nadu.

2.1. Preparation of extracts

The leaves of *glycosmis cochinchinensis* were shade dried and pulverized in a mechanical grinder. The powder (500g) was successively extracted with various solvents such as methanol, ethanol, water by using maceration technique for 24 hours. The extracts were concentrated under reduced pressure in a rotary evaporator after concentrated, the extract were stored in desiccators for further use. It was stored in desiccator throughout the period of investigation without the risk of spoilage or deterioration. The various extracts were designated as follows the metanol extract of leaves of *glycosmis cochinchinensis* as (MEGC), ethanol extract of leaves of *glycosmis cochinchinensis* as (EEGC) and aqueous extract of leaves of *glycosmis cochinchinensis* as (AQGC)

2.2. Qualitative Phytochemical Analysis

All the extracts (0.05 g/ml) were subjected to preliminary phytochemical screening following standard methods [12-13] for detection of the following constituents.

2.2.1. Steroids

Five milliliters of chloroform and 5 ml of H2O4 were added to 500 µl of the prepared plant extracts. The presence of steroids was indicated by a colour change from violet to blue or green or a ring of blue/green or if the upper layer turns red and the sulphuric layer was yellow with a green fluorescence.

2.2.2. Saponins

About 3 ml of plant extracts were added to 3 ml of distilled water and shaken vigorously. The formation of a stable persistent froth was taken as a positive test for saponins.

2.2.3. Alkaloids

Approximately 3 ml of extracts were added to 3 ml of 1% HCl and heated for 20 min. The mixtures were then cooled and used to perform the following tests: Mayer's test To the filtrate in test tube I, 1 ml of Mayer's reagent was added drop by drop. The formation of a greenish coloured or cream precipitate indicated the presence of alkaloids.

2.2.4. Dragendoff's test

To the filtrate in test tube II, 1 ml of Dragendoff's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

2.2.5. Wagner's test

To the filtrate in tube III, 1 ml of Wagner's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

2.2.6. Protein Xanthoproteic test

A few drops of nitric acid were added to 2 ml of plant extracts and a colour change to yellow was observed.

2.2.7. Anthocyanin

Approximately 2 ml of the prepared plant extracts were added to 2 ml of 2N HCl and ammonia. The appearance of a pink red coloration that turned blue violet indicated the presence of anthocyanin.

2.2.8. Coumarin

About 3 ml of 10% NaOH were added to 2 ml of plant extracts. The formation of a yellow colour was an indication for the presence of coumarins.

2.2.9. Carbohydrates Fehling test

Two milliliters of each plant extract were hydrolyzed with dilute HCl, neutralized with alkali, and then heated with Fehling's solution A and B. The formation of a red precipitate was an indication for the presence of a reducing sugar.

2.2.10. Flavonoid

Alkaline reagent test Three milliliters of plant extract was treated with 1 ml of 10% NaOH solution. The formation of an intense yellow colour was an indication of the presence of flavonoids.

2.2.11. Leucoanthocyanin

Approximately 5 ml of isoamyl alcohol were added to 5 ml of plant extracts. The appearance of a red upper layer indicated the presence of leucoanthocyanin.

2.2.12. Cardiac Glycosides Keller-Killani Test

Two milliliters of plant extract were treated with 2 ml glacial acetic acid containing a drop of FeCl3. A brown coloured ring or brown-violet under a brown greenish layer indicated the presence of cardiac glycosides.

2.2.13. Phlobatannins

Two milliliters of 1% HCl were added to 3 ml of plant extracts and boiled. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

2.2.14. Terpenoids

Approximately 2 ml of chloroform and 3 ml of H2O4 were added to 5 ml of plant extracts. A reddish-brown coloration was taken as positive test for terpenoids.

2.2.15. Test for phenols and tannins

Ferric chloride test

Two milliliters of 5% solution of $FeCl_3$ were added to 1 ml crude extracts. A black or blue-green colour indicated the presence of tannins and phenols

2.3. Determination of antioxidant activity

2.3.1. DPPH radical scavenging assay

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams *et al*[14-15] with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract. Corresponding blank sample were prepared and BHT was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. Decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\text{Inhibition \%} = Ac\text{-}As/Ac\times100$$

Where Ac is the absorbance of the control As is the absorbance of the sample

2.4. Results and Discussion

2.4.1. Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Agilent Resolutions Pro), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

The FTIR spectrum of leaf extracts (prepared in different solvents) of *glycosmis cochinchinensis* is given in Fig 2. The characteristic absorption band were exhibited at 2974 cm^{-1} (for C-H stretching), 1461 cm^{-1} (for C-H bending) for C-H group and at 1700 cm^{-1} for carbonyl groups (C=O) were exhibited by extract.

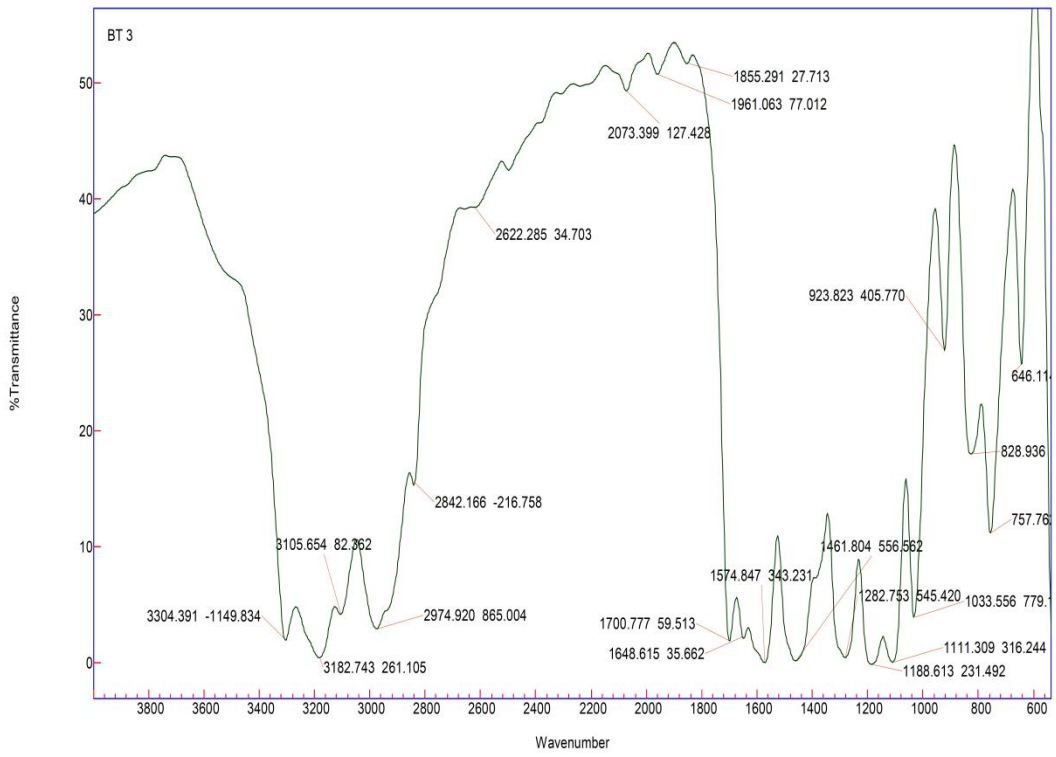

Figure 2: IR spectral studies of plant leaf extract of *glycosmis cochinchinensis*

2.5. Phytochemical analysis

Table-1. shows that the results of the qualitative screening of phytochemical analysis from *glycosmis cochinchinensis*. Flavonoids, steroids, tannins, glycosides, alkaloids and reducing sugars are present in methanolic extracts. But protein is present in ethanol extract only. These phytochemical compounds are known to support bioactivity. Thus responsible for the antioxidant activities.

Table 1: Preliminary phytochemical screening of various leaves extracts of ***glycosmis cochinchinensis***.

Phytochemicals	Solvents		
	Methanol	**Ethanol**	**Water**
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	-	-	-
Phenols	+	+	+
Steroids	+	+	-
Tannins	+	+	+
Carbohydrates	+	+	+
Protein	-	+	-
Glycosides	+	+	-

2.6. Antioxidant activity

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. The Table 2 shows the antioxidant activities of the methanol, ethanol and water extracts of plant leaf extract assessed using the DPPH radical scavenging. 50- 250 µg/ml of methanol extracts produced moderate to high DPPH scavenging activity in experimental plants. The highest DPPH scavenging activity was observed (95.71 %) at the concentration of 250µg/ml . At a concentration of 250µg/ml, the scavenging activity of ethanol extract of the leaves reached 92.56%, while at the same concentration, that of the water extract shows 92.88%.

Table 2: DPPH scavenging activities of the plant leaf extracts of the of *glycosmis cochinchinensis*

Conc.of extracts (µg/ml)	% Inhibition			
	BHT	**Methanol**	**Ethanol**	**Water**
50	79.84 ±0.12	82.02 ±0.65	73.66 ±0.74	72.88 ±0.24
100	83.25 ±0.56	85.43 ±0.87	79.29 ±0.89	77.51 ±0.04
150	88.44 ±0.28	91.34 ±0.94	85.32 ±0.35	84.33 ±0.82
200	94.21 ±0.08	93.25 ±0.65	89.47 ±0.63	89.75 ±0.91
250	97.14 ±0.97	95.71 ±0.14	92.56 ±0.58	92.88 ±0.42

Though the DPPH radical scavenging abilities of the extracts were less than those of BHT (97.14) at 250µg/ml, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants[16-19]. In the present experiment, plant extract contain flavonoid and tannin and all the plants extract showing antioxidant property , this nature may be due to tannins and flavonoids.

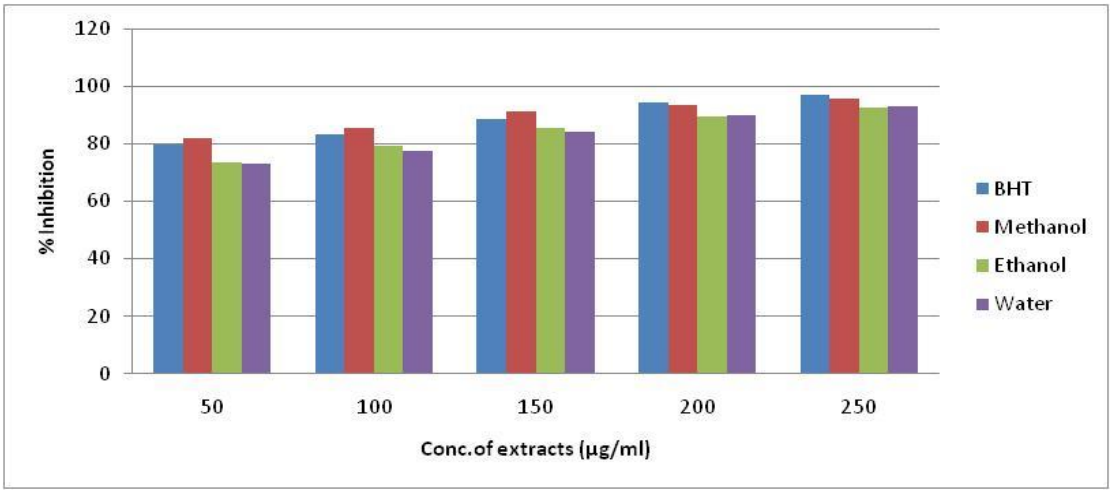

Figure 3. DPPH scavenging activities of the plant leaf extracts of the of *glycosmis cochinchinensis*.

2.7. Conclusion

The present study suggests that qualitative phytochemical screening of crude leaf extracts of glycosmis cochinchinensis supports the presence of bioactive compounds such as Flavonoids, steroids, terpeniods, tannins, glycosides, alkaloids reducing sugars and Phenols, alkaloids and tanin in the medicinal plant and thus responsible for the antioxidant activities. Higher antioxidant property of methanolic extract than aqueous extracts might be due to greater solubility of active constituents in ethanol than in water. So, these extracts could be used as new sources of natural antioxidants for treatment of oxidative stress induced diseases.

Reference

1. Duraipandiyan, V., Ayyanar, M., Ignacimuthu, S., BMC complementary and Alternative medicine, Vol.6, October 2006, P.35-41.
2. Aruoma, O.I., Journal of the American Oil Chemists Society.1998; volume 75, Issue 2, Pp199-212.
3. Aruoma, O.I., Halliwell. B., Biochemical Journal 1987; Dec.15; 248(3), 973-976.
4. Kris-Etherton. P.M., Lefevre. M., Beecher, G.R., Gross. M.D., Keen. C.L., Etherton. T.D. Annual Review of Nutrition; 2004: Vol 24, 511-538.
5. Repetto. M.G, Llesuy.S.f.,. Brazilian Journal of Medicine and Biological Research. 2002; Braz J Med Biol Res, May 2002, Volume 35(5) 523-534.
6. Marchioli R, Schweiger C, Levantesi G, Tavazzi L, and Valagussa F. Int J of Pharm Sci Rev and Res. 2001; 36:53-63.
7. Roja.G., Rao.C.,. Journal of Herbs.Spices and Medicinal Plants2000; 7.71-102.
8. Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimepoulou AN, Boskou D. Food Chem. 2006; 94, 19-25.

9. Surat Laphookhieo et al., Phytochemistry Letters 6 (2013) 337–339.

10. Zahira Yaakob, Ehsan Ali, Afifi Zainal, Masita Mohamad, Mohd Sobri Takriff: 2014 An overview: biomolecules from microalgae for animal feed and aquaculture.
11. Keller, 1986; Keller & Bzdek, *1984*; Meehan *et al*. *Chakra* pendulum assessment scores for change in all seven *chakras.* 1990;
12. *Sofowora A. Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan. 1993; pp 150.*
13. *Trease G.E., and Evans, W.C. Pharmacognosy. 13th edn. Bailliere Tindall, London, 1989, pp 176- 180.*
14. *Brand-Williams W, Cuvelier ME, Berset C. Lebensmittel Wissenschaft und Technologie 1995; 28: 25-30.*
15. Ayoola GA, Sofidiya T, Odukoya O, Coker H.A.B J. Pharm. Sci. & Pharm. Pract. 2006; 8: 133-136.
16. Martinez AC, Marcelo EL, Marco AO, Moacyr M. Plant Science 2001; 160: 505-515.
17. Dasgupta N, De B.. Food Chemistry 2004; 88: 219-224.
18. Beauchamp C, Fridovich I Analytical Biochemistry 1971; 44: 276-287.
19. Chung SK, Osawa T, Kawakishi S. Biosci Biothech Biochem 1997; 61: 118-123.